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By Leah Ann Howard

Entitled
A NOVEL FURANONE-CONTAINING ANTIBACTERIAL SYSTEM FOR IMPROVED DENTAL RESTORATIVES

For the degree of Master of Science in Biomedical Engineering

Is approved by the final examining committee:

Dong Xie
Chair

Hiroki Yokota

Richard Gregory

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Approved by Major Professor(s): Dong Xie

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A NOVEL FURANONE-CONTAINING ANTIBACTERIAL SYSTEM FOR IMPROVED DENTAL RESTORATIVES

A Thesis
Submitted to the Faculty
of
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by
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of
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ABSTRACT

Howard, Leah Ann. M.S.B.M.E., Purdue University, May 2012. A Novel Furanone-Containing Antibacterial System for Improved Dental Restoratives. Major Professor: Dong Xie.

The furanone derivatives and their constructed polymers were synthesized, characterized and formulated into dental glass-ionomer cement (GIC) and resin composite for improved antibacterial properties. Compressive strength (CS) and *S. mutans* viability were used to evaluate the mechanical strength and antibacterial activity of the restoratives. Fuji II LC cement and P60 were used as control. The specimens were conditioned in distilled water at 37 ^{o}C for 24 h prior to testing. The effects of loading, saliva and aging on CS and *S. mutans* viability were investigated. The antibacterial effect of the furanone derivative on other bacteria was also studied.

Chapter 2 describes how we studied and evaluated the formulated antibacterial glass-ionomer cement by incorporating the synthesized furanone derivative-containing polymer into the formulation. The results show that all the formulated furanone-containing cements showed a significant antibacterial activity, accompanying with an initial CS reduction. Increasing loading significantly enhanced antibacterial activity but reduced the initial CS of the formed cements. The derivative showed a broad antibacterial spectrum on bacteria including *S. mutans, lactobacillus, S. aureus* and *S. epidermidis*. Human saliva did not affect the antibacterial activity of the cement. The long-term aging study indicates that the cements may have a long-lasting antibacterial function.

Chapter 3 describes how we studied and evaluated the formulated antibacterial resin composite by incorporating the synthesized furanone derivative into the basic resin formulations. The results show that the modified resin composites showed a significant antibacterial activity without substantially decreasing the mechanical strengths. With 5 to 30% addition of the furanone derivative, the composite kept its original CS unchanged but showed a significant antibacterial activity with a 16-68% reduction in the *S. mutans* viability. Further, the antibacterial function of the new composite was not affected by human saliva. The aging study indicates that the composite may have a long-lasting antibacterial function.

In summary, we have developed a novel furanone-containing antibacterial system for dental restoratives. Both glass-ionomer cement and resin composite have demonstrated significant antibacterial activities. The modified experimental glass-ionomer cement is a promising system because the reduced strength of the cement with addition of the furanone-containing polymer is still above those demonstrated by original commercial cement Fuji II LC. The modified resin composite shows nearly no reduction in mechanical strength after incorporation of the antibacterial furanone derivative. It appears that both experimental cement and resin composite are clinically attractive dental restoratives that can be potentially used for long-lasting restorations due to their high mechanical strength and permanent antibacterial function.

INTRODUCTION

1.1 Background

Long-lasting restoratives and restoration are clinically attractive because they can reduce patents' pain and expense as well as the number of their visits to dental offices [1-4]. In dentistry, both restorative materials and oral bacteria are believed to be responsible for the restoration failure [2]. Secondary caries is found to be the main reason to the restoration failure of dental restoratives including resin composites and glass-ionomer cements [1-4]. Secondary caries which often occur at the interface between the restoration and the cavity preparation is primarily caused by demineralization of tooth structure due to invasion of plaque bacteria (acid-producing bacteria) such as *Streptococcus mutans* (*S. mutans*) in the presence of fermentable carbohydrates [4]. To make long-lasting restorations, the materials should be made antibacterial. Although numerous efforts have been made on improving antibacterial activities of dental restoratives, most of them have been focused on release or slow-release of various incorporated low molecular weight antibacterial agents such as antibiotics, zinc ions, silver ions, iodine and chlorhexidine [5-9]. Yet release or slow-release can lead or has led to a reduction of mechanical properties of the restoratives over time, short-term effectiveness, and possible toxicity to surrounding tissues if the dose or release is not properly controlled [5-9]. Materials containing quaternary ammonium salt (QAS) groups have been studied extensively as an important antimicrobial material and used for a variety of applications due to their potent antimicrobial activities [10-14]. These materials are found to be capable of killing bacteria that are resistant to other types of cationic antibacterials [15]. The examples of the QAS-containing materials as antibacterials for dental restoratives include incorporation of a methacryloyloxydodecyl pyridinium bromide as an antibacterial monomer into resin composites [12], use of

methacryloxylethyl cetyl ammonium chloride as a component for antibacterial bonding agents [16, 17], addition of quaternary ammonium polyethylenimine nanoparticles into composite resins [18, 19], and incorporation of PQAS into GICs [20]. All of these studies found that the QAS-containing materials did exhibit significant antibacterial activities. However, it has been reported that human saliva can significantly decrease the antibacterial activity of the QAS-containing restoratives, probably due to electrostatic interactions between QAS and proteins in saliva [21, 22]. Recently, furanone derivatives have been found to have strong antitumor [23, 24] and antibacterial functions [25]. Therefore, we would like to explore them in dental applications.

1.2. Hypothesis and Objectives

It is our hypothesis that incorporating the synthesized furanone derivative or derivative-containing polymer into experimental dental resin composite and/or glass-ionomer cement would provide a novel route for formulation of novel antibacterial dental restoratives. Commercial Fuji II LC, recently developed experimental high-strength cement and resin composite were used as controls. The objectives of the study in this thesis were to:

(1) synthesize and characterize the furanone derivative and/or derivative-containing polymer
(2) formulate the GIC and resin composite with the furanone derivatives
(3) evaluate the mechanical strengths of the formed restoratives
(4) evaluate the antibacterial activity of the formulated restoratives.

Chapter 2 mainly describes the synthesis, characterization, formulation and evaluation of the GICs composed of the furanone-containing polymer. Chapter 3 mainly describes the synthesis, characterization, formulation and evaluation of the resin composites composed of the furanone derivative. The effects of loading, saliva, and aging time on compressive strength and *S. mutans* viability were also investigated.

2. PREPARATION AND EVALUATION OF A NOVEL FURANONE-CONTAINING ANTIBACTERIAL GLASS-IONOMOER DENTAL RESTORATIVE

2.1 Introduction

A furanone derivative and its constructed polyacid were synthesized, characterized and formulated into the experimental high-strength cements. Compressive strength (CS) and *S. mutans* viability were used to evaluate the mechanical strength and antibacterial activity of the cements. All the formulated furanone-containing cements showed a significant antibacterial activity, accompanying with an initial CS reduction. The effect of the furanone derivative loading was significant. Increasing loading significantly enhanced antibacterial activity but reduced the initial CS of the formed cements. The derivative showed a broad antibacterial spectrum on bacteria including *S. mutans, lactobacillus, S. aureus* and *S. epidermidis*. Human saliva did not affect the antibacterial activity of the cement. The long-term aging study indicates that the cements may have a long-lasting antibacterial function. Within the limitations of this study, it appears that this experimental antibacterial cement is a clinically attractive dental restorative due to its high mechanical strength and antibacterial function.

2.2 Materials and Methods

2.2.1 Materials

Acrylic acid (AA), glycolic acid (GA), 3,4-dichloro-5-hydroxy-2(5H)-furanone (DHF), dipentaerythritol, 2-bromoisobutyryl bromide (BIBB), 2,2'-azobisisobutyronitrile (AIBN), triethylamine (TEA), CuBr, N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA), dl-camphoroquinone (CQ), 2-(dimethylamino)ethyl methacrylate (DMAEMA), pyridine, tert-butyl acrylate (t-BA), glycidyl methacrylate (GM), hydrochloric acid (HCl, 37%), sulfuric acid, pyridine, diethyl ether, dioxane, N,N-dimethylformamide (DMF), toluene, hexane and tetrahydrofuran (THF) were used as received from Sigma-Aldrich Co. (Milwaukee, WI) without further purifications. Light-cured glass-ionomer cement Fuji II LC and Fuji II LC glass powders were used as received from GC America Inc (Alsip, IL).

2.2.2 Synthesis and Characterization

2.2.2.1 Synthesis of 3,4-dichloro-5-(1,3-glycerol ethylene glycolate methacrylate)-2-furanone (DGEGM)

To a solution containing DHF (0.5 mol), toluene and sulfuric acid (1% by mole), glycolic acid (0.55 mol) in toluene was added. The reaction was run at 90-100 ^{o}C for 3-4 h until no more water came out. Then toluene was removed using a rotary evaporator. The formed *3,4-dichloro-5-(hydroxyacetic acid)-2-furanone (DHAF)* was purified by washing with sodium bicarbonate and distilled water. After freeze-drying, DHAF (0.5 mol) was mixed with GM (0.52 mol) and pyridine (3%, by weight) in DMF. The mixture was reacted at 50 ^{o}C for 8h [27], followed by washing with hexane and diethyl ether and drying in a vacuum oven prior to use. The synthesis scheme is shown in Fig. 2.1, part A.

2.2.2.2 Synthesis of the Poly(acrylic acid-co-DGEGM)

The linear poly(acrylic acid-co-DGEGM) or poly(AA-co-DGEGM) was prepared following our published procedures [26]. Briefly, to a flask containing a solution of AA (0.1 mol) and DGEGM (0.1 mol) in THF, AIBN (1% by mole) in THF was added. After the reaction was run under N_2 purging at 60 ^{o}C for 18 h, the polymer was precipitated with diethyl ether, followed by drying in a vacuum oven. The synthesis scheme is shown in Figure 2.1, part B.

2.2.2.3 Synthesis of the GM-Tethered Star-Shape Poly(acrylic acid)

The GM-tethered 6-arm star-shape poly(acrylic acid) (PAA) was synthesized similarly as described in our previous publication [28]. Briefly, dipentaerythritol (0.06 mol) in 200 ml THF was used to react with BIBB (0.48 mol) in the presence of TEA (0.35 mol) to form the 6-arm initiator. t-BA (0.078 mol) in 10 ml dioxane was then polymerized with the 6-arm initiator (1% by mole) at 120 °C in the presence of CuBr (3%)-PMDETA (3%) catalyst complex via ATRP. The resultant 6-arm poly(t-BA) was hydrolyzed with HCl and dialyzed against distilled water. The purified star-shape PAA was obtained via freeze-drying, followed by tethering with GM (50% by mole) in DMF in the presence of pyridine (1% by weight) [28]. The GM-tethered star-shape PAA was recovered by precipitation from diethyl ether, followed by drying in a vacuum oven at room temperature. The scheme for the structure of the 6-arm star-shape PAA is also shown in Figure 2.1, part C.

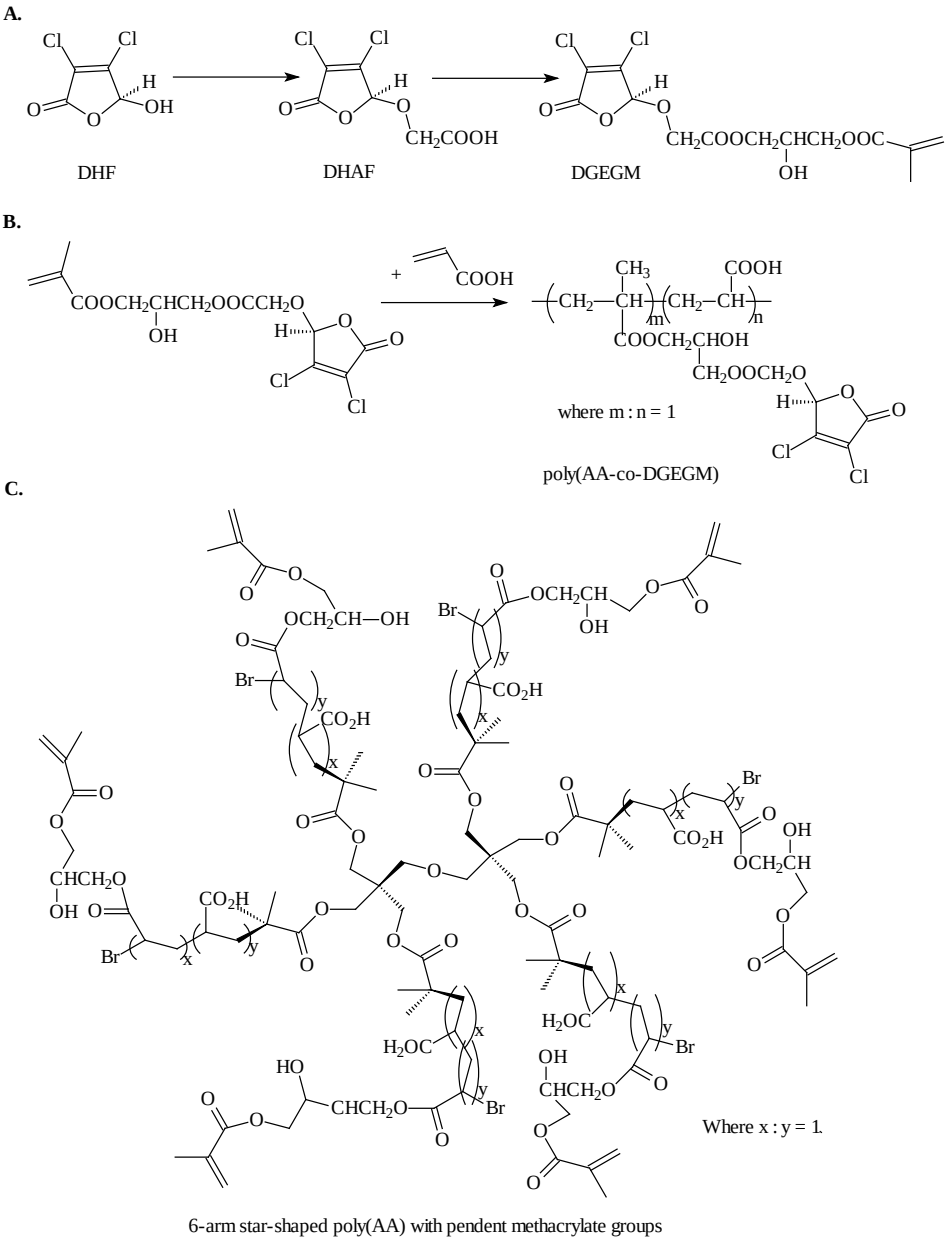


Figure 2.1 Synthesis schematic diagram for: A. DGEGM synthesis, B. poly(AA-co-DGEGM) synthesis, C. structure of the 6-arm star-shaped poly(AA) with pendent methacrylates.

2.2.2.4 Characterization

The chemical structures of the synthesized furanone derivatives were characterized by Fourier transform-infrared (FT-IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The proton NMR (^{1}HNMR) spectra were obtained on a 500 MHz Bruker NMR spectrometer (Bruker Avance II, Bruker BioSpin Corporation, Billerica, MA) using deuterated dimethyl sulfoxide and chloroform as solvents and FT-IR spectra were obtained on a FT-IR spectrometer (Mattson Research Series FT/IR 1000, Madison, WI).

2.3 Evaluation

2.3.1 Cement Sample Preparation for Strength and Antibacterial Tests

The experimental cements were formulated with a two-component system (liquid and powder) [28]. The liquid was formulated with the light-curable star-shape PAA, water, 0.9% CQ (photo-initiator, by weight) and 1.8% DC (activator). The polymer/water (P/W) ratio = 70:30 (by weight). Fuji II LC glass powder was either used alone or mixed with the synthesized poly(AA-co-DGEGM) to formulate the cements, where the poly(AA-co-DGEGM) loading ratio = 1, 3, 5, 7, 10, or 20% (by weight) of the glass and the P/L ratio = 2.7 (by weight).

Specimens were fabricated at room temperature according to the published protocol [28]. Briefly, the cylindrical specimens were prepared in glass tubing with dimensions of 4 mm in diameter by 8 mm in length for compressive strength (CS), 4 mm in diameter by 2 mm in length for diametral tensile strength (DTS), and 4 mm in diameter by 2 mm in depth for antibacterial tests. The rectangular specimens were prepared in a split Teflon mold with dimensions of 3 mm in width by 3 mm in thickness by 25 mm in length for flexural strength (FS) test. All the specimens were exposed to blue light (EXAKT 520 Blue Light Polymerization Unit, EXAKT Technologies, Inc., Oklahoma

City, OK) for 2 min, followed by conditioned in 100% humidity for 15 min, removed from the mold and conditioned in distilled water at 37 ^{o}C for 24 h unless specified, prior to testing.

2.3.2 Strength Measurements

CS, DTS, and FTS were performed on a screw-driven mechanical tester (QTest QT/10, MTS Systems Corp.). The crosshead moved at a speed of 1mm/minute. The FS test was performed with three-point bending with a span of 20 mm between each support. In order to obtain a mean value for each material or formulation, six to eight specimens were tested. . CS was calculated using an equation of $CS = P/\pi r^2$, where P = the load at fracture and r = the radius of the cylinder. DTS was determined from the relationship $DTS = 2P/\pi dt$, where P = the load at fracture, d = the diameter of the cylinder, and t = the thickness of the cylinder. FS was obtained using the expression $FS = 3Pl/2bd^2$, where P = the load at fracture, l = the distance between the two supports, b = the breadth of the specimen, and d = the depth of the specimen.

2.3.3 Antibacterial Test

For the antibacterial test published procedures were followed closely [20]. The main bacteria used to evaluate the antibacterial activity of the glass ionomer was *S. mutans*. Three other strains of bacteria; *lactobacillus, Staphylococcus aureus (S. aureus),* and *Staphylococcus epidermidis (S. epidermidis),* were used to demonstrate and evaluate the broad antibacterial activity of the glass ionomer material.

One or two colonies of *S. mutans* (or the other bacteria) from an agar plate were suspended in 5 mL of tryptic soy broth (TSB) for 24 hours and incubated at 37°C. Material specimen samples were pretreated with ethanol for approximately 10 seconds, and then incubated with the bacteria in TSBS (TSB supplemented with 1% sucrose) at 37°C for 48 hours under 5% CO_2. The specimens were then taken out and the bacteria

and TSBS mixture was vortexed and sonicated to break up any bacterial clumps. Equal volumes of the red and green dyes from the LIVE/DEAD BacLight bacterial viability kit L7007 from Molecular Probes, Inc. were combined and mixed for 1 minute. Next, 3 µL of the dye mixture was added to 1 mL of the bacteria suspension, mixed by vortexing for 10 seconds, sonicated for 10 seconds, and then vortexed for another 10 seconds. The mixture was kept in the dark for about 15 minutes prior to analysis. Finally, 20 L of the stained bacterial suspension was put onto a microscope slide and analyzed using the Nikon Microphot-FXA fluorescent microscope. Triple replica was used to obtain a mean value for each material.

2.3.4 Saliva Effect Test

Human saliva was obtained from a healthy volunteer, and the collected saliva was centrifuged for 15 minutes at 12,000g to remove debris [21]. The resulting supernatant from the centrifuged saliva was filtered with a 0.45 µm sterile filter. The filtrate was stored in 1 ml aliquots in a -20°C freezer prior to use.

The sterilized cement specimen was incubated in a small tube containing 1 ml of saliva at 37°C for 2 hours. The specimen was then taken out of the saliva and used for antibacterial testing in the same procedures as described in section 2.3.3.

2.3.5 Aging of the Specimens

In order to analyze the effect of aging on the specimens, those that would be used for both CS and antibacterial testing were conditioned in distilled water at 37°C for 1 day, 3 days, 7 days, and 30 days. After being aged in the water, the procedure for testing follows the same steps as described in sections 2.3.2 and 2.3.3.

2.3.6 Statistical Analysis

One-way analysis of variance (ANOVA) with the post hoc Tukey-Kramer multiple-range test was used to determine significant differences of mechanical strength, antibacterial and pulp cell viability tests among the materials or formulations in each group. A level of $\alpha = 0.05$ was used for statistical significance.

2.4 Results

2.4.1 Characterization

Table 2.1 shows the FT-IR peaks (cm^{-1}) for GA, DHF, DHAF, GM and DGEGM. The disappearance of the peak at 3362 for pseudo hydroxyl group on pseudo ester and appearance of the two new peaks at 1810 and 1768 for the carbonyl groups from pseudo ester and GA ester as well as a broad COOH peak at 3650-2500 confirmed the formation of DHAF. The disappearance of the broad carboxylic acid peak at 3650-2500 and appearance of 3480 (OH), 3001-2930 (CH_3), 1786, 1742 and 1720 (C=O from pseudo ester, GA ester and methacrylate), and 1638 and 1620 (C=C on methacrylate and pseudo ester) confirmed the formation of DGEGM. Table 2.2 shows the ^{1}HNMR chemical shifts (ppm) for GA, DHF, DHAF, GM and DGEGM. All the new chemical shifts shown in Table 2.2 for DGEGM confirmed the formation of DGEGM.

2.4.2 Evaluation

Figures 2.2 (a and b) show the effect of the DGEGM-containing polymer content on CS and *S. mutans* viability of the cements. Both the mean CS and S. mutans viability values decreased with an increase in the furanone-containing polymer content. There were no statistically significant differences between 1% and 3% for CS and between 0% and 1%, 1% and 3%, and 7% and 10% for the S. mutans viability ($p>0.05$). The DGEGM addition showed a reduction of 19-75% for CS and 3-50% for the *S. mutans* viability.

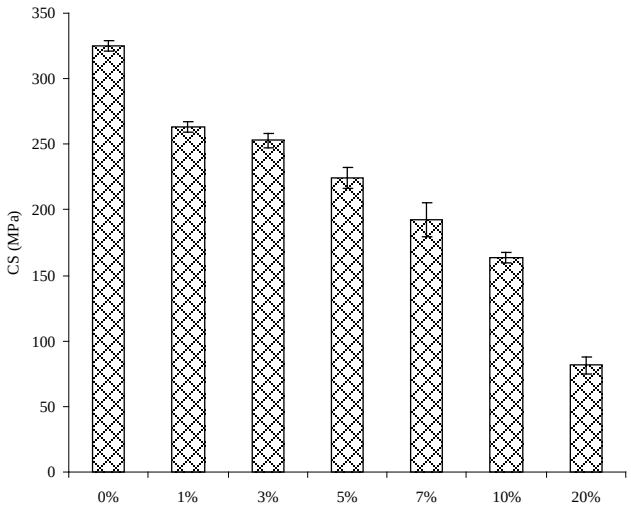
Table 2.1. The characteristic peaks from the FT-IR spectra

Material	The characteristic peaks (cm^{-1})
GA	3480 (O-H stretching on -OH), 3650-2500 (broad –OH stretching on –COOH), 1731 (C=O stretching on carbonyl group), 1646 (C=O stretching on carbonyl group due to strong intramolecular hydrogen-bonded hydroacid), 1352 and 993 (C-O-C stretching), 1434 and 1227 (O-H deformation on OH), 1086, 884 and 661 (C-O stretching)
DHF	3362 (O-H stretching on OH), 1766 (C=O stretching on carbonyl group), 1644 (C=C stretching on internal C=C), 1332, 1027, 951 and 778 (C-O-C stretching on pseudo ester), 1451 and 1237 (O-H deformation on pseudo OH), 1279, 1118, 889 and 602 (C-O stretching on pseudo C-OH), 746 (C-Cl stretching)
DHAF	3650-2500 (broad –OH stretching on carboxylic acid), 1810 and 1768 (C=O stretching on carbonyl groups of both pseudo ester and GA ester), 1640 (C=C stretching on internal C=C), 1429 (C-H deformation on –C=C- group), 1325, 1233, 1027, 951 and 778 (C-O-C stretching on pseudo ester), 1278, 1118 and 887 (C-O stretching on pseudo C-OH), 744 (C-Cl stretching)
GM	3075 (asymmetrical C-H stretching), 3001-2930 (-CH_3), 1722 (C=O stretching on carbonyl group), 1637 (C=C stretching), 1486, 1403, 1255, 1196, 908, 843, 815 and 654 (-CH, -CH_2 and ring deformation, stretching and other vibrations on epoxy ring), 1453, 1379 and 1349 (C-H deformation on –C=C- group), 1317, 1296, 1077, 944, 762 and 654 (C-O and C-O-C stretching on ester)
DGEGM	3480 (O-H stretching on newly formed OH), 3001-2930 (-CH_3 on methacrylate), 1786, 1742 and 1720 (C=O stretching on carbonyl groups of pseudo ester, GA ester and methacrylate), 1638 and 1620 (C=C stretching on methacrylate and internal C=C), 1511 (C-O-C deformation on newly formed ester), 1458, 1381 and 1169 (C-H deformation on –C=C- group), 1446 and 1417 (O-H deformation on newly formed OH), 891 and 841 (C-O deformation and stretching on newly formed OH), 1319, 1233, 1017, 951 and 775 (C-O-C stretching on pseudo ester), 1297, 1104 and 653 (C-O stretching on carbonyl and OH groups), 747 (C-Cl stretching)

Table 2.2. The characteristic chemical shifts from the ^{1}HNMR spectra

Material	The characteristic chemical shifts (ppm)
GA	11.55 (-COOH), 4.57 (-CH_2-) and 3.90 (-OH)
DHF	6.25 (-CH) and 3.45 (-OH)
DHAF	11.55 (-COOH), 6.25 (-CH) and 4.90 (-CH_2-)
GM	6.10 and 5.70 (H_2C=C-), 5.45 (-CH), 3.2-3.3 (-CH_2-), 2.65-2.8 (-CH_2-) and 1.90 (-C=CH-)
DGEGM	6.20 (-CH), 6.10 and 5.70 (H_2C=C-), 4.90 (-CH_2- on GM), 4.15 (-CH_2- on GM), 3.70 (-CH- on GM), 3.50 (-CH_2- on GA), 3.25 (-OH) and 1.90 (-C=CH-)

a.



b.

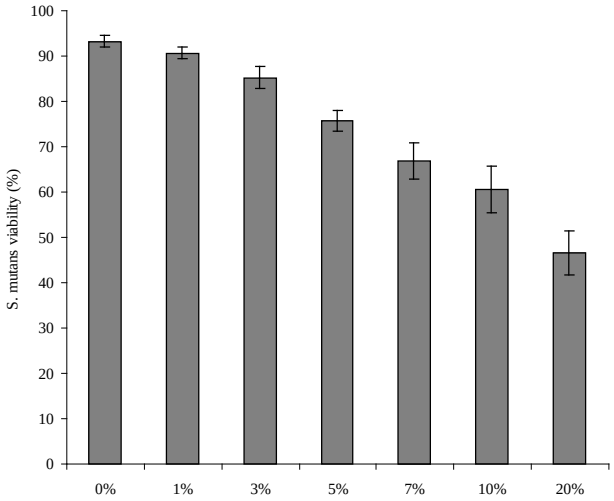


Figure 2.2. Effect of the poly(AA-co-DGEGM) content on CS and *S. mutans* viability of the experimental cements:
a. Effect on CS;
b. Effect on the S. mutans viability. MW of the 6-arm poly(acrylic acid) = 17,530 Daltons; Filler = Fuji II LC or Fuji II LC + poly(AA-co-DGEGM); Grafting ratio = 50%; P/L ratio = 2.7; P/W ratio = 70:30; For CS, specimens were conditioned in distilled water at 37 ^{o}C for 24 h prior to testing. For S. mutans viability, specimens were conditioned in distilled water at 37 ^{o}C for 24 h, followed by incubating with S. mutans before antibacterial testing.

Figure 2.3 shows the effect of the DGEGM-containing cement aging on CS and *S. mutans* viability. After 30-day aging in water, the cement showed a significant CS increase (26%) for 7% DGEGM addition. However, no statistically significant changes were found in the *S. mutans* viability for the cement.

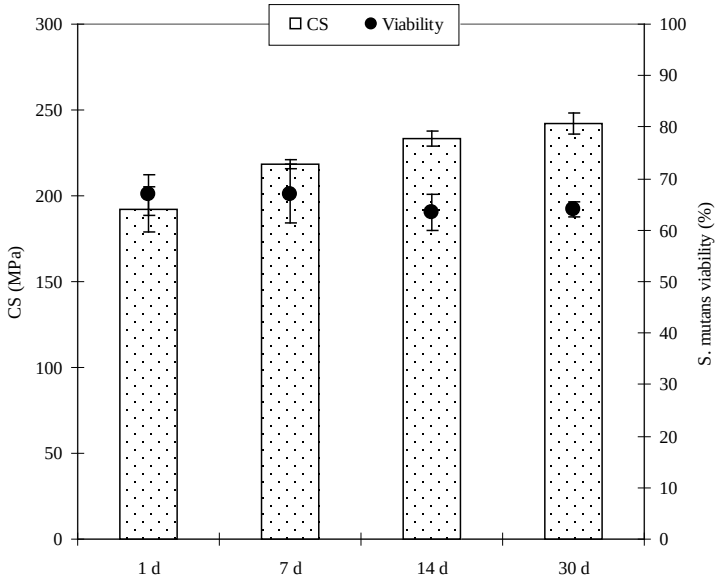


Figure 2.3. Effect of aging on CS and *S. mutans* vialibility of the experimental cements: The formulations were the same as those described in Figure 2.2, except for the poly(AA-co-DGEGM) content = 7%. Specimens were conditioned in distilled water at 37 ^{o}C for 1, 7, 14 and 30 days prior to testing.

Figure 2.4 shows the effect of the DGEGM-containing polymer content on the viability of four bacteria including *S. mutans*, *lactobacillus*, *S. aureus* and *S. epidermidis*. Increasing DGEGM decreased the viability of all the bacteria, where there were no statistically significant differences in viability between 7% and 10% for S. mutans, between 5% and 7% for lactobacillus and between 10% and 20% for S. epidermidis ($p > 0.05$). With 20% of DGEGM addition, the viability of all four bacteria decreased to 47%.

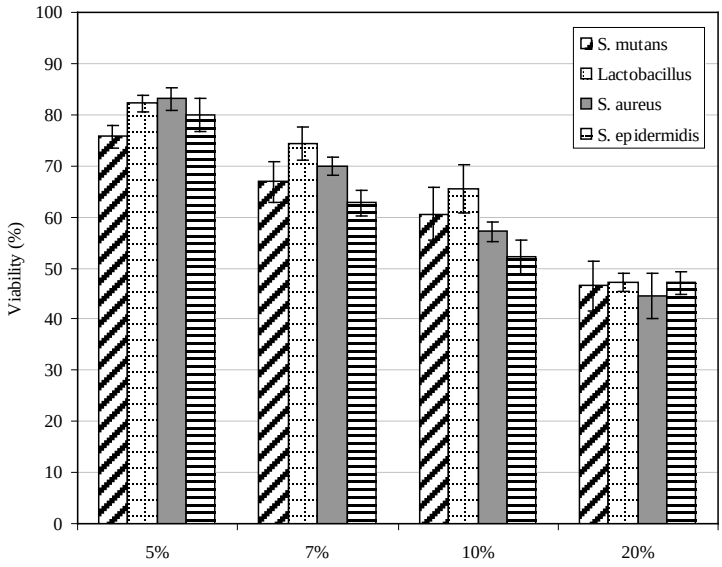


Figure 2.4. Effect of the poly(AA-co-DGEGM) content on the viability of different bacteria after culturing with the experimental cements: The formulations were the same as those described in Figure 2.2. Specimens were conditioned in distilled water at 37 ^{o}C for 24 h, followed by incubating with bacteria before antibacterial testing.

Figure 2.5 shows the effect of human saliva on the *S. mutans* viability after culture with the antibacterial cements. There were no statistical differences in the viability found between cements with and without human saliva treatment.

Table 2.3 shows the property comparison among the cements with 0%, 5% and 7% of DGEGM addition. As compared to the cement (0% DGEGM), the cements with 5% and 7% DGEGM showed a decrease in all the measured strengths. The yield strength (YS), compressive modulus (M), CS, diametral tensile strength (DTS), flexural strength (FS) showed 16-27%, 3-11%, 31-41%, 13-20% and 29-32% decreases, respectively, among which CS and FS showed more reductions. The *S. mutans* viability also showed a significant decrease with 19-28% reduction.

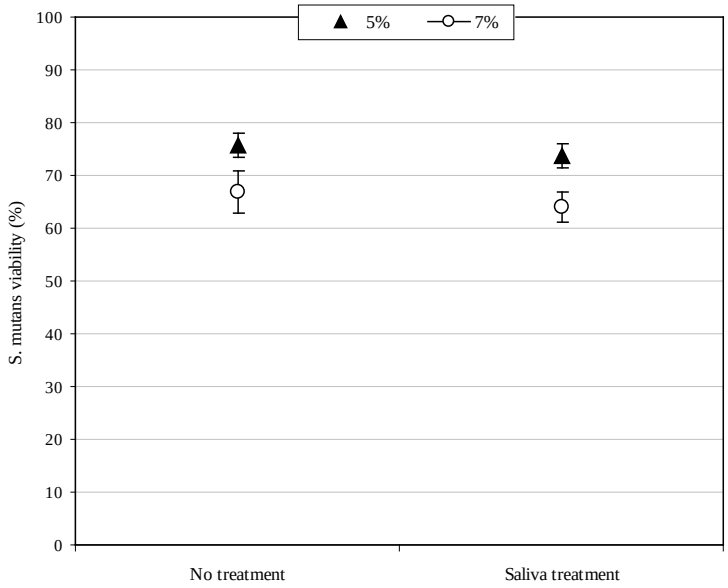


Figure 2.5. Effect of human saliva on the *S. mutans* viability after culturing with the cements: The formulations were the same as those described in Figure 2.2. Specimens were soaked in human saliva at 37 ^{o}C for 2 h, followed by incubating with *S. mutans* before antibacterial testing.

Table 2.3. Comparison of properties of the experimental cements with and without poly(AA-co-DGEGM)[1]

Polymer (%)[2]	YS[3] [MPa]	M[4] [GPa]	CS [MPa]	DTS[5] [MPa]	FS[6] [MPa]	Viability (%)
0	170.1 (5.6)[7]	7.12 (0.12)	325.3 (4.2)	60.1 (0.9)	87.2 (2.2)	93.2 (1.3)
5	142.3 (6.9)	6.86 (0.17)	224.7 (7.9)	52.2 (2.3)[a]	62.1 (3.3)[b]	75.7 (2.2)
7	124.6 (5.9)	6.31 (0.05)	192.5 (13)	48.3 (1.1)[a]	58.9 (4.6)[b]	66.9 (4.0)

[1]The formulations were the same as those described in Figure 2.2; [2]Polymer = poly(AA-co-DGEGM) (%, by weight); [3]YS = CS at yield; [4]M = compressive modulus; [5]DTS = diametral tensile strength; [6]FS = flexural strength; [7]Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different ($p > 0.05$). Specimens were conditioned in distilled water at 37 ^{o}C for 24 h, followed by direct testing for all the strengths and incubating with *S. mutans* for 48 h for antibacterial testing.

2.5 Discussion

Furanone-containing materials are reported to have a broad range of biological and physiological properities, including antitumor, antibiotic, haemorrhagic, fungicidal, and insecticidal activities[23-25]. Although it is known that furanones do have these antibacterial properties, the biological mechanism in how these derivatives work is still under investigation [25]. To explore the application of these compounds in dental research, we synthesized a novel furanone rerivative and applied it to dental GICs. The following discussion demonstrates how the newly synthesized DGEGM was incorporated into our experimental GICs and its effect on the mechanical and antibacterial properties of the formed cements.

Figure 2.2 shows the effect of the DGEGM-containing polymer content on CS and *S. mutans* viability of the cements. It is apparent that with DGEGM addition the cement showed a decrease in CS and S. mutans viability. The cements lost 19-75% of its original CS value (325 MPa) with 1-20% DGEGM addition, among which the cements with 5 and 7% DGEGM showed a 30-40% loss but the values were still close to 200 MPa. The loss of CS can be attributed to the incorporated DGEGM because hydrophobic DGEGM did not contribute any strength enhancement to the cements. Regarding the S. mutans viability, DGEGM significantly increased the antibacterial activity of the cement. With 1-20% DGEGM addition, the S. mutans viability was reduced from 3 to 50%, among which the cements with 5 and 7% DGEGM showed a 19-28% reduction. Considering the feasibility of GICs applied in dental clinics, the CS value below 200 MPa may not be well acceptable because the CS values of most commercially available light-cured GICs are in the range of 180-240 MPa [5, 29]. Therefore, the cements with 5 and 7% DGEGM addition were chosen to evaluate other properties.

It is well known that GICs increase their strengths with time due to constant salt-bridge formations [30]. To confirm if the DGEGM-modified GIC still follows the pattern that most GICs have, we examined both CS and antibacterial function of the cements after aging in water for 1 day, 7 days, 14 days and 30 days. The result in Fig. 3 shows that

the cements with 7% DGEGM showed 26% increase in CS after 30-day aging in water and no changes in the *S. mutans* viability were found. The reason can be attributed to the fact that the DGEGM-containing polymer is a copolymer of acrylic acid and DGEGM. It is known that the carboxylic acid group plays a key role in GIC setting and salt-bridge formation. The DGEGM-containing polymer not only provided antibacterial function but also supplied carboxyl groups for salt-bridge formation. The latter helped the polymer firmly attached to the glass fillers. The results also imply that the DGEGM-containing polymer did not leach out of the cement.

As stated before, *lactobacillus* is another main oral cavity-producing bacterium although it is not as popular as *S. mutans. S. aureus* and *S. epidermidis* are two major bacteria that often cause skin and other implant infections. To examine the antibacterial activity of DGEGM on these bacteria, we compared the viability of all the four bacteria after incubating with the cements. The result in Figure 4 shows that no statistically noticeable differences in viability were found among the four bacteria, even though the absolute values were different from one another. Increasing the DGEGM-containing polymer content significantly decreased the viability of all the bacteria, indicating that the DGEGM-containing polymer is a polymer with a broad antibacterial spectrum.

Figure 2.5 shows the effect of human saliva on the *S. mutans* viability after culturing with the DGEGM –containing cements. No statistically significant differences in the *S. mutans* viability were found between the cements with and without human saliva treatment. It has been noticed that saliva can significantly reduce the antibacterial activity of the QAS or PQAS-containing materials based on the mechanism of contact inhibition [21, 22]. The reduction was attributed to the interaction between positive charges on QAS or PQAS and amphiphilic protein macromolecules in saliva, thus leading to formation of a protein coating which covers the antibacterial sites on QAS or PQAS [21, 22]. Unlike QAS or PQASA, DGEGM does not carry any charges. That may be why the DGEGM-modified cements did not show any reduction in antibacterial activity after treating with saliva.

Finally we compared YS, M, CS, DTS, FS and the *S. mutans* viability of the cements having 0, 5 and 7% of DGEGM. As shown in Table 2.3, the DGEGM-modified cements were 16-17% in YS, 3.6-11% in modulus, 31-41% in CS, 13-20% in DTS and 29-32% in FS lower than the cement without DGEGM addition. On the other hand, the DGEGM-modified cements were much higher (19 and 28% higher) in antibacterial activity than the cement without DGEGM addition.

2.6 Conclusions

The dental restorative material developed and evaluated is a novel furanone-containing antibacterial glass-ionomer cement. All of the furnanone-modified cements show significant antibacterial activity, along with an initial reduction in CS strength. DGEGM loading had a significant effect on both antibacterial and mechanical strength. With an increase in loading, the cement had enhanced antibacterial activity, but an initial reduced CS. Modifying the cement with DGEGM showed a broad range of antibacterial activity, killing *S. mutans, lactobacillus, S. aureus,* and *S. epidermidis*. When coated with human saliva, the antibacterial power of the cement was not affected. The long-term aging study indicated that the cements have a long-lasting antibacterial function. Because of the antibacterial function and strength properties, it appears that the furanone-containing glass ionomer is a clinically attractive dental restorative.

3. PREPARATION AND EVALUATION OF A NOVEL FURANONE-CONTAINING ANTIBACTERIAL GLASS-IONOMOER DENTAL RESTORATIVE

3.1 Introduction

A novel furanone-containing antibacterial resin composite has been prepared and evaluated. Compressive strength (CS) and *S. mutans* viability were used to evaluate the mechanical strength and antibacterial activity of the composites. The modified resin composites showed a significant antibacterial activity without substantially decreasing the mechanical strengths. With 5 to 30% addition of the furanone derivative, the composite kept its original CS unchanged but showed a significant antibacterial activity with a 16-68% reduction in the *S. mutans* viability. Further, the antibacterial function of the new composite was not affected by human saliva. The aging study indicates that the composite may have a long-lasting antibacterial function. Within the limitations of this study, it appears that the experimental antibacterial resin composite may potentially be developed into a clinically attractive dental restorative due to its high mechanical strength and antibacterial function.

3.2 Materials and Methods

3.2.1 Materials

Bisphenol A glycerolate dimethacrylate (BisGMA), Bisphenol A ethoxylate dimethacrylate (BisEMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), dl-camphoroquinone (CQ), 2-(dimethylamino)ethyl methacrylate (DMAEMA), sulfuric acid, toluene, acryloyl chloride (AC), 3,4-dichloromalealdehydic acid (DA), ethyl acetate and sodium bicarbonate were used as received from Sigma-Aldrich Co. (Milwaukee, WI) without further purifications. The untreated glass fillers from Herculite XRV (0.7 microns) were used as received from Sybron Dental Specialties (Newport Beach, CA). Filtek P-60 resin composite was used as received from 3M ESPE (St. Paul, MN).

3.2.2 Synthesis and Characterization

3.2.2.1. Synthesis of 5-acryloyloxy-3,4-dichlorocrotonolactone (AD)

To a solution containing DA (0.5 mol) and toluene, AC (0.52 mol) in toluene was added. After the mixture was run at 90-100 ^{o}C for 3-4 h, toluene was removed using a rotary evaporator. The residue was then washed with sodium bicarbonate and distilled water, followed by extracting with ethyl acetate. The formed AD was obtained by completely removing ethyl acetate using the rotary evaporator before drying in a vacuum oven. The synthesis scheme is shown in Figure 3.1.

3.2.2.2. Characterization

The chemical structure of the synthesized AD and starting chemicals was characterized by Fourier transform-infrared (FT-IR) spectroscopy and nuclear magnetic

resonance (NMR) spectroscopy. The proton NMR (^{1}HNMR) spectra were obtained on a 500 MHz Bruker NMR spectrometer (Bruker Avance II, Bruker BioSpin Corporation, Billerica, MA) using deuterated dimethyl sulfoxide and chloroform as solvents and FT-IR spectra were obtained on a FT-IR spectrometer (Mattson Research Series FT/IR 1000, Madison, WI).

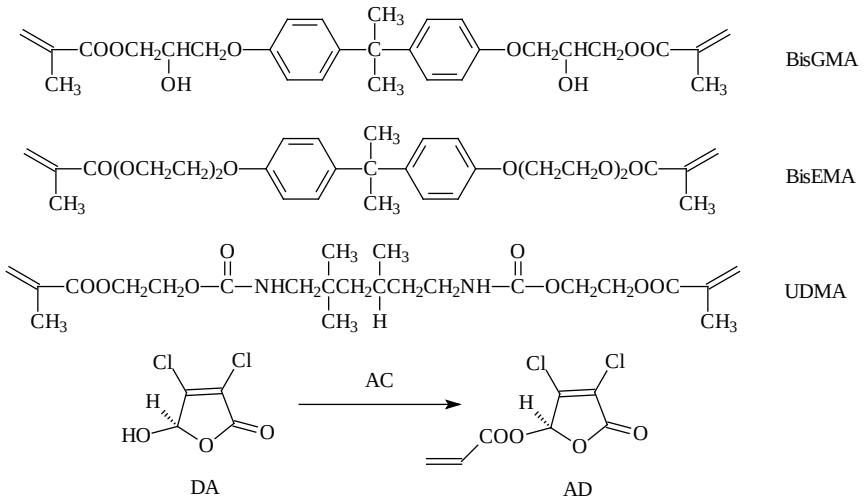


Figure 3.1. Schematic diagram for the structures of the oligomers used in the study and synthesis of AD from the reaction of DA with AC.

3.3 Evaluation

3.3.1 Cement Sample Preparation for Strength and Antibacterial Tests

The experimental resin composites were formulated with a two-component system (liquid and powder) [20]. The liquid was formulated with the newly synthesized monomer AD, BisGMA, UDMA, BisEMA, CQ (photo-initiator) and DMAEMA (activator). AD was mixed with a mixture of BisGMA, UDMA and BisEMA (BisGMA/UDMA/BisEMA = 1:1:1, by weight) in a ratio of AD/a mixture = 0, 5, 10, 20, 30, 40, 50 and 70% (by weight) unless specified. CQ (1.0% by weight) and DMAEMA (2.0%) were added for photo-

initiation. TEGDMA was also used as reference in evaluating viscosities of the formulated liquids. The untreated glass Herculite XRV (0.7 microns) powders were used as fillers and treated with γ-(trimethoxysilyl)propyl methacrylate, following the published protocol [20]. A filler level at 75% (by weight) was used throughout the study unless specified.

Specimens were fabricated by thoroughly mixing the liquid with the treated fillers at room temperature according to the published protocol [20]. Briefly, the cylindrical specimens were prepared in glass tubing with dimensions of 4 mm in diameter by 8 mm in length for compressive strength (CS), 4 mm in diameter by 2 mm in length for diametral tensile strength (DTS), and 4 mm in diameter by 2 mm in depth for antibacterial tests. The rectangular specimens were prepared in a split Teflon mold with dimensions of 3 mm in width by 3 mm in thickness by 25 mm in length for flexural strength (FS) test. All the specimens were exposed to blue light (EXAKT 520 Blue Light Polymerization Unit, EXAKT Technologies, Inc., Oklahoma City, OK) for 2 min, followed by removing from the mold prior to testing.

3.3.2 Strength Measurements

CS, DTS and FS tests were performed on a screw-driven mechanical tester (QTest QT/10, MTS Systems Corp., Eden Prairie, MN), with a crosshead speed of 1 mm/min. The FS test was performed in three-point bending with a span of 20 mm between supports. Six to eight specimens were tested to obtain a mean value for each material or formulation in each test. CS was calculated using an equation of $CS = P/\pi r^2$, where P = the load at fracture and r = the radius of the cylinder. DTS was determined from the relationship $DTS = 2P/\pi dt$, where P = the load at fracture, d = the diameter of the cylinder, and t = the thickness of the cylinder. FS was obtained using the expression $FS = 3Pl/2bd^2$, where P = the load at fracture, l = the distance between the two supports, b = the breadth of the specimen, and d = the depth of the specimen.

3.3.3 Other Property Determinations

The viscosity of the formulated liquid was determined at 23 ^{o}C using a cone/plate viscometer (RVDV-II+CP, Brookfield Eng. Lab. Inc., MA, USA), as described elsewhere [26]. For exotherm measurement, the heat generated from the setting reaction of the resin composite was determined with a slightly modified ASTM F-451 procedure [26]. Briefly, the composite paste was placed in a cylindrical Teflon mold with dimensions of 30 mm in diameter by 6 mm in height and covered with a Teflon plunger having holes for allowing the excessive paste to escape. A digital thermocouple (Fisher Scientific, Springfield, NJ) was inserted into the center of the resin composite and used to record the temperature change. The peak temperature was defined as the exotherm. The polymerization shrinkage was determined using an equation of % Shrinkage = $(1 - d_{uncured}/d_{cured}) \times 100$, where d_{cured} is the density of cured composite and $d_{uncured}$ the density of uncured composite [33]. The densities of the uncured and cured composites were determined by weighing the uncured composite paste injected from a calibrated syringe which was used to determine the volume of the uncured paste and weighing the cured cylindrical specimen whose volume was measured in a calibrated buret in the presence of water, respectively. The mean values were averaged from three readings.

3.3.4 Antibacterial Test

The antibacterial test was conducted following the published procedures [31]. *S. mutans* was used to evaluate the antibacterial activity of the studied composites. Briefly, one or two colonies of *S. mutans* were suspended in 5 mL of tryptic soy broth (TSB. Specimens pretreated with ethanol (10 s) were incubated with *S. mutans* in TSB, supplemented with 1% sucrose, at 37°C for 48 h under 5% CO_2. After equal volumes of the red and the green dyes (LIVE/DEAD BacLight bacterial viability kit L7007, Molecular Probes, Inc., Eugene, OR, USA) were combined in a microfuge tube and mixed thoroughly for 1 min, 3 μL of the dye mixture was added to 1 mL of the bacteria suspension, mixed by vortexing for 10 s, sonicating for 10 s as well as vortexing for another 10 s, and kept in dark for about 15 min, prior to analysis. Then 20 μL of the

stained bacterial suspension was analyzed using a fluorescent microscope (Nikon Microphot-FXA, Melville, NY, USA). Triple replica was used to obtain a mean value for each material.

3.3.5 Saliva Effect Test

Human saliva, obtained from a healthy volunteer, was centrifuged for 15 min at 12,000g to remove debris [21]. After the supernatant was filtered with a 0.45-μm sterile filter, the filtrate was stored in a -20 ^{o}C freezer prior to testing. The sterilized composite specimen (see 2.3.4) was incubated in a small tube containing 1 ml of saliva at 37 ^{o}C for 2 h [21], followed by placing in 5 ml TSB supplemented with 1% sucrose. The rest of the procedures for antibacterial test are described in 3.3.4.

3.3.6 Aging of the Specimens

The specimens for both CS and antibacterial activity aging tests were conditioned in distilled water at 37 ^{o}C for 1 day, 3 days, 7 days and 30 days, followed by direct testing for CS (see 3.3.2 for details) and incubating with *S. mutans* for 48 h for antibacterial testing (see 3.3.4 for details).

3.3.7 Statistical Analysis

One-way analysis of variance (ANOVA) with the post hoc Tukey-Kramer multiple-range test was used to determine significant differences of mechanical strength and antibacterial tests among the materials or formulations in each group. A level of $\alpha = 0.05$ was used for statistical significance.

3.4 Results

3.4.1 Characterization

Figure 3.2 shows the FT-IR peaks (cm^{-1}) for GA, DHF, DHAF, GM and DGEGM. The disappearance of the peak at 3362 for pseudo hydroxyl group on pseudo ester and appearance of the two new peaks at 1810 and 1768 for the carbonyl groups from pseudo ester and GA ester as well as a broad COOH peak at 3650-2500 confirmed the formation of DHAF. The disappearance of the broad carboxylic acid peak at 3650-2500 and appearance of 3480 (OH), 3001-2930 (CH_3), 1786, 1742 and 1720 (C=O from pseudo ester, GA ester and methacrylate), and 1638 and 1620 (C=C on methacrylate and pseudo ester) confirmed the formation of DGEGM. Figure 3.3 shows the ^{1}HNMR chemical shifts (ppm) for GA, DHF, DHAF, GM and DGEGM. All the new chemical shifts shown in Table 3.2 for DGEGM confirmed the formation of DGEGM.

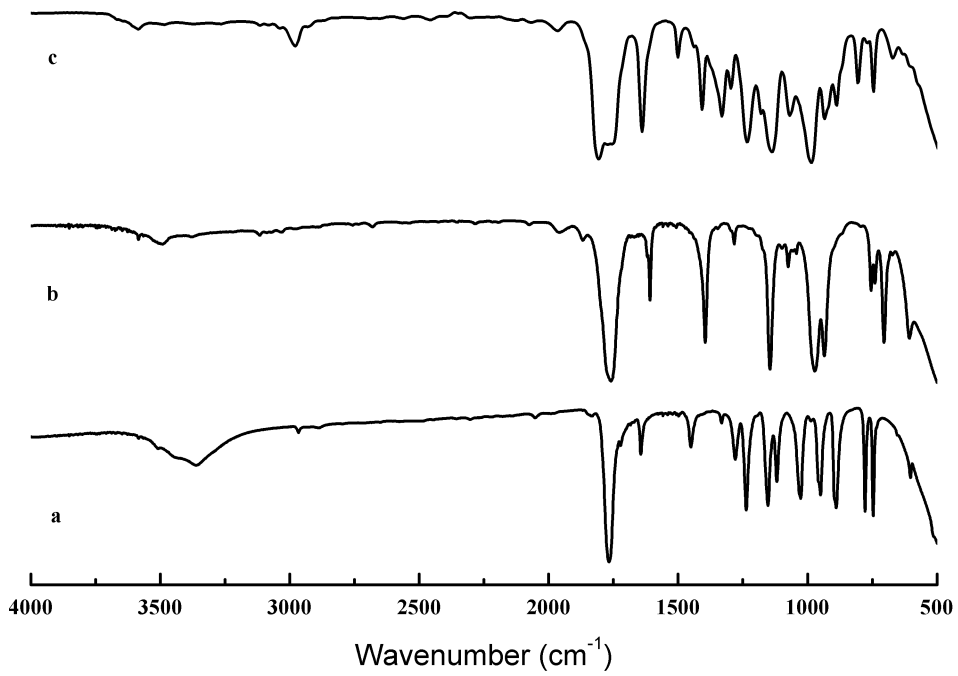


Figure 3.2. FT-IR spectra for DA, AC and AD: (a) DA; (b) AC and (c) AD.

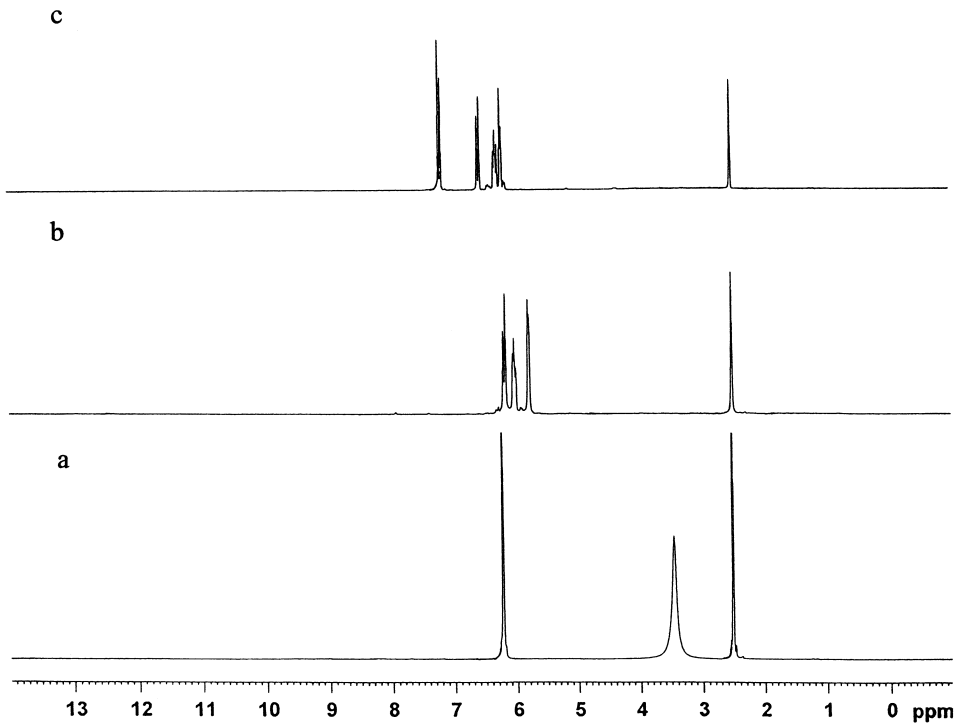


Figure 3.3. ^{1}HNMR spectra for DA, AC and AD: (a) DA; (b) AC and (c) AD.

3.4.2 Evaluation

Table 3.1 shows the effects of different oligomer mixtures and filler loading on compressive strengths of the experimental resin composites and on viscosity of the resin liquid. For the effect of oligomer mixtures, the mean strength value was in the decreasing order of: YS (MPa): BisGMA/UDMA > BisGMA/TEGDMA > BisGMA > BisGMA/UDMA/BisEMA > BisGMA/BisEMA; M (GPa): BisGMA/TEGDMA > BisGMA/UDMA > BisGMA > BisGMA/UDMA/BisEMA > BisGMA/BisEMA; CS (MPa): BisGMA/TEGDMA > BisGMA/UDMA/BisEMA > BisGMA/UDMA > BisGMA = BisGMA/BisEMA; Viscosity (cp): BisGMA >> BisGMA/UDMA > BisGMA/BisEMA > BisGMA/UDMA/BisEMA > BisGMA/TEGDMA. For the effect of filler loading, YS: 3.0 > 2.3 > 3.3 > 2.7; M: 3.0 > 3.3 > 2.7 > 2.3; CS: 3.0 > 3.3 > 2.7 > 2.3.

Table 3.1. Effects of different oligomer mixtures and filler loading on compressive strengths of the composites

	YS[3] [MPa]	M[4] [GPa]	CS [MPa]	Viscosity[5] (cp)
Effect of oligomer[1]				
BisGMA/BisEMA	83.8 (7.7)[6]	4.53 (0.06)	284.8 (15)[e]	528
BisGMA/TEGDMA	133.1 (9.2)[a]	7.01 (0.16)[c]	302.6 (18)[e]	345
BisGMA/UDMA	139.9 (12)[a]	6.88 (0.05)[c]	290.4 (16)[e]	882
BisGMA/UDMA/BisEMA	115.4 (6.1)[b]	5.89 (0.19)[d]	297.4 (15)[e]	478
BisGMA	118.5 (10)[b]	6.12 (0.07)[d]	285.6 (9.3)[e]	N/D
Effect of filler loading[2]				
2.3	108.2 (10)[f]	5.13 (0.34)[g]	275.9 (12)[i]	384
2.7	103.1 (4.0)[f]	5.18 (0.21)[g]	279.9 (12)[i]	384
3.0	121. 5 (18)	6.14 (0.53)[h]	317.0 (13)	384
3.3	105.5 (6.1)[f]	6.05 (0.22)[h]	293.4 (8.4)	384

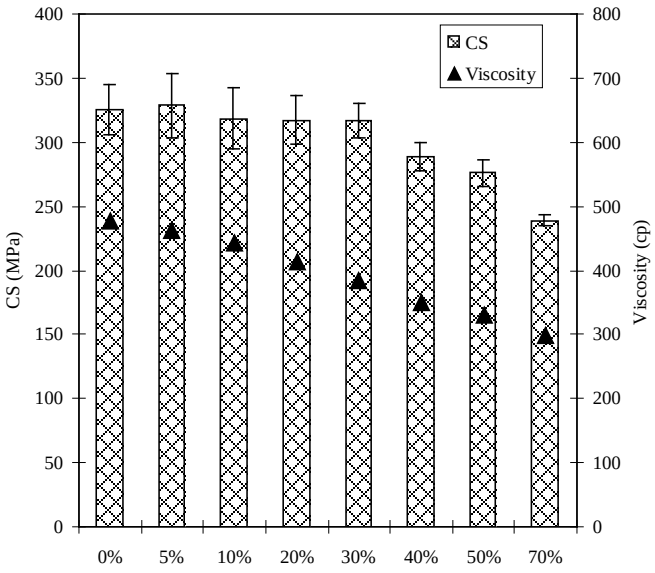
[1]The liquid oligomers were mixed in 1:1 and 1:1:1 ratios (by weight) without AD addition; The filler/resin ratio = 3.0 or 75% (by weight). [2]The resin was composed of AD, BisGMA, UDMA and BisEMA, where AD = 30% and BiSGMA/UDMA/BisEMA = 1:1:1; [3]YS = CS at yield; [4]M = compressive modulus; [5]Viscosity was determined from the liquid resin only and N/D = not determined due to the extremely high viscosity of BisGMA; [6]Entries are mean values with standard deviations in parentheses and the mean values with the same letter in each category were not significantly different ($p > 0.05$). Specimens were directly used for CS testing.

Figures 3.4 (a and b) show the effect of the AD content on CS and *S. mutans* viability of the experimental composites. For CS (Figure 4a), the mean CS value (MPa) was in the decreasing order of 5% > 0% > 10% = 20% = 30% > 40% > 50% > 70%, where there were no statistically significant differences among 0%, 5%, 10%, 20% and 30% and between 40% and 50% ($p > 0.05$). The AD addition did not change the CS of the composites until reaching 40%. From 40% to 70%, CS decreased 11-27% of its original value. The viscosity value was in the decreasing order of 0% > 5% > 10% > 20% > 30% > 40% > 50% > 70%. For the *S. mutans* viability (Fig. 4b), increasing the AD content significantly decreased the *S. mutans* viability. The mean viability values were decreased from 82 to 1% with 5 to 70% AD addition and all the values were significantly different from each other ($p < 0.05$).

Figure 3.5 shows the effect of human saliva on the *S. mutans* viability after culturing with the experimental composites. No statistically significant differences in the *S. mutans* viability were found between the experimental composites with and without human saliva treatment.

Figures 3.6 (a and b) show the effect of the composite aging in water on CS and *S. mutans* viability. For CS (Figure 6a), After 7-day aging in water, all the composites with AD addition showed no statistically significant differences in CS from each other ($p > 0.05$), regardless filler loading or aging time. For the *S. mutans* viability (Figure 3.6b), no statistically significant differences ($p > 0.05$) were found after 7-day aging at the same filler loading, although a slight increase was noticed.

a.



b.

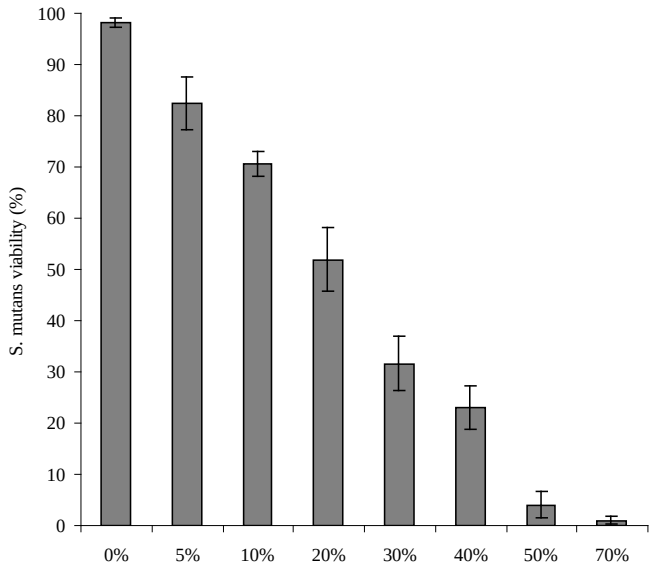


Figure 3.4. Effect of the AD content on the viscosity of the resin liquid formulations and CS as well as *S. mutans* viability of the experimental composites:
a. Effect on CS and viscosity;
b. Effect on the *S. mutans* viability. AD content (%, by weight) = AD/(AD/BisGMA/UDMA/BisEMA), where BiSGMA/UDMA/BisEMA = 1:1:1; The filler/resin ratio = 3.0 or 75% (by weight). For CS, specimens were directly used for the testing. For the *S. mutans* viability, specimens were incubated with *S. mutans* before antibacterial testing.

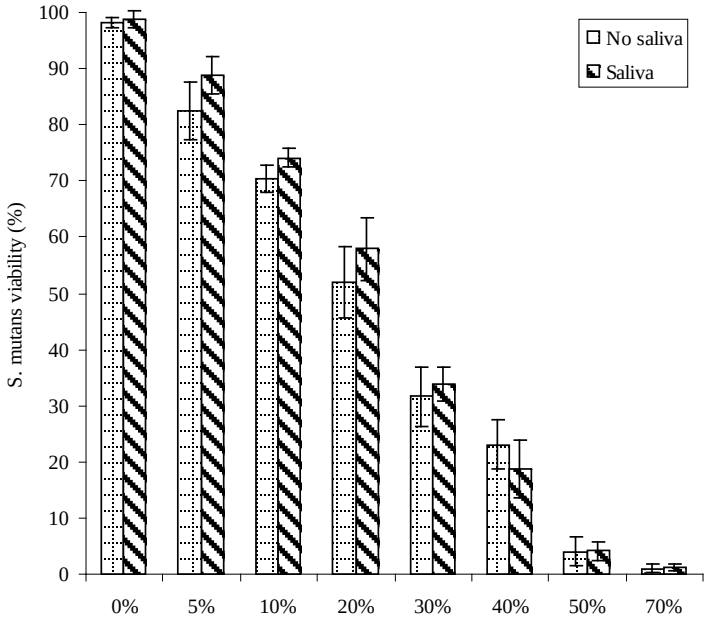
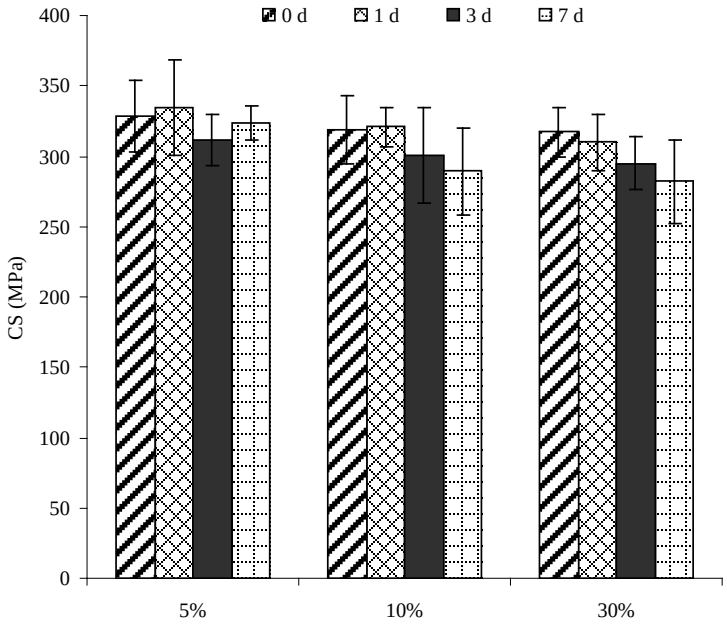


Figure 3.5. Effect of human saliva on the *S. mutans* viability after culturing with the composites: The formulations were the same as those described in Figure 3.4. Specimens were soaked in human saliva at 37 ^{o}C for 2 h, followed by incubating with *S. mutans* before antibacterial testing.

a.



b.

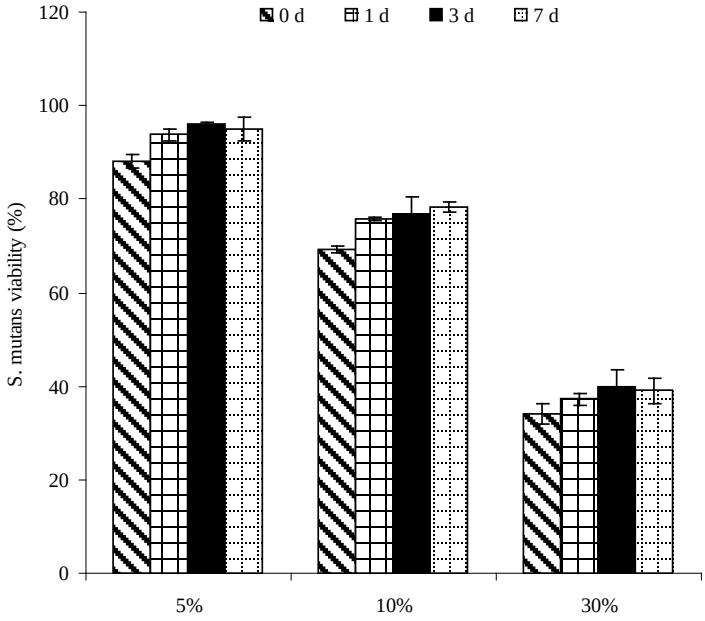


Figure 3.6. Effect of aging on CS and *S. mutans* viability of the experimental composites:
a. Effect on CS;
b Effect of aging on CS and *S. mutans* viability of the experimental composites. The formulations were the same as those described in Figure 3.4. Specimens were conditioned in distilled water at 37 ^{o}C for 1, 3 and 7 days prior to testing.

Table 3.2 shows a property comparison of the resin composites with and without AD addition. The mean values for yield strength (YS), compressive modulus (M), CS, diametral tensile strength (DTS) and flexural strength (FS) were in the decreasing order of: YS (MPa): 5% > 0% > 10% > 30%, where 0% and 5% were not statistically significantly different from each other ($p > 0.05$); M (GPa): 5% > 0% > 10% > 30%, where 0% and 5% were not statistically significantly different from each other ($p > 0.05$); CS (MPa): 0% = 5% = 10% = 30% ($p > 0.05$); DTS (MPa): 0% > 5% > 10% > 30%, where 0%, 5% and 10% were not statistically significantly different from one another; FS (MPa): 0% > 5% > 10% > 30%, where 5% and 10% were not statistically significantly different from each other ($p > 0.05$). The mean values for shrinkage, exotherm and *S. mutans* viability were in the decreasing order of: Shrinkage (%): 30% > 10% > 5% > 0%; Exotherm (^{o}C): 30% > 10% > 5% > 0%; *S. mutans* viability (%): 0% > 5% > 10% > 30%.

Table 3.2. Comparison of properties of the resin composites with and without AD addition[1]

AD (wt %)	YS [MPa]	M [GPa]	CS [MPa]	DTS[2] [MPa]	FS[3] [MPa]	Shrinkage (%)	Exotherm (°C)	Viability (%)
0	141.0 (6.7)[a, 4]	6.99 (0.04)[b]	325.1 (19)[c]	61.1 (1.9)[d]	123.9 (5.6)	2.09 (0.23)	2.7	98.3 (0.8)
5	151.2 (8.5)[a]	7.03 (0.07)[b]	328.6 (21)[c]	59.1 (2.1)[d]	106.9 (5.4)[e]	2.36 (0.15)	3.1	82.4 (5.2)
10	136.7 (5.4)	6.68 (0.12)	318.5 (22)[c]	55.8 (1.4)[d]	101.5 (5.6)[e]	3.23 (0.25)	3.5	70.5 (2.4)
30	115.4 (6.0)	5.89 (0.19)	317.0 (13)[c]	47.3 (5.4)	82.1 (3.9)	4.99 (0.32)	4.2	31.6 (9.0)
P-60[5]	157.2 (3.8)	7.44 (0.14)	348.5 (11)	71.2 (0.9)	139.3 (4.2)	1.34 (0.10)	2.3	97.2 (1.1)

[1]The formulations were the same as those described in Figure 3.4. [2]DTS = diametral tensile strength; [3]FS = flexural strength; [4]Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different ($p > 0.05$); [5]P-60 = commercial resin composite. Specimens were directly used for all the property testing without aging.

3.5 Discussion

To explore the application of these furanone compounds in dental research, we synthesized a photocurable furanone rerivative and applied it to dental resin composites. The following discussion demonstrates how the newly synthesized AD was incorporated into a dental composite formulation and its effect on some mechanical, physical and antibacterial properties of the formed dental composite.

It is known that commercially available dental resin composites usually contain 70-80% inorganic fillers and 20-30% of a liquid mixture of BisGMA and TEGDMA or BisGMA with one or two other oligomers including UDMA, BisEMA and TEGDMA [5]. In formulating the liquid for resin composites, viscosity is one of the very important criteria [5] because it determines what percentage of fillers can be incorporated and what properties the formed system might have. During the study, we found that the newly synthesized AD has a low viscosity (154 cp), which is just a little bit higher than that for TEGDMA (132 cp). To avoid having unexpected low viscosity values and mimic commercial resin composite systems, we decided to make a new formulation that contains AD while eliminating TEGDMA (commonly used in commercial resin composites) [5]. The oligomers including BisGMA, BisEMA and UDMA were used to formulate the liquid. While formulating the liquid, we also tested the corresponding CS of the formulated composite. As shown in Table 1, the mixture of BisGMA/UDMA/BisEMA showed a higher CS and appropriate viscosity value (478 cp). With this viscosity, the liquid mixture could be used to formulate with AD without changing the viscosity significantly, as compared to the liquid formulation used in commercial resin composites (BisGMA/TEGDMA = 345 cp). In fact, the measured viscosity values for the liquid formulations composed of AD, BisGMA, BisEMA and UDMA (BisGMA/UDMA/BisEMA = 1:1:1) were in the range of 463 to 298 cp corresponding to 5-70% AD addition (see Figure 4a).

In order to determine how much fillers should be incorporated into the resin, we evaluated the effect of the filler content on CS and found that the filler/liquid ratio of 3.0

or 75% showed the highest YS, modulus and CS values (see Table 1). Thus we chose the mixture of BisGMA/UDMA/BisEMA and 75% filler to formulate the antibacterial resin composites.

Next, we evaluated the effect of the AD content on CS and *S. mutans* viability of the experimental composites. From the results in Fig. 4a, increasing AD did not decrease the CS of the composites until reaching 40%. From 40 to 70%, CS decreased 11-27% of its original value. For the *S. mutans* viability (Fig. 4b), increasing the AD content significantly decreased the *S. mutans* viability. The mean viability values were decreased from 82 to 1% with 5 to 70% AD addition. Apparently with 30% AD addition, the resin composite showed the best antibacterial properties without decreasing CS. In other words, the resin composite with 30% AD seemed to be the optimal formulation for this new antibacterial dental composite.

Figure 3.5 shows the effect of human saliva on the *S. mutans* viability after culturing with the experimental composites. No statistically significant differences in the *S. mutans* viability were found between the composites with and without human saliva treatment. It has been noticed that saliva can significantly reduce the antibacterial activity of the QAS or PQAS-containing materials based on the mechanism of contact inhibition [21, 22]. The reduction was attributed to the interaction between positive charges on QAS or PQAS and amphiphilic protein macromolecules in saliva, thus leading to formation of a protein coating which covers the antibacterial sites on QAS or PQAS [21, 22]. Unlike QAS or PQASA, AD does not carry any charges. That may be why the AD-modified resin composites did not show any reduction in antibacterial activity after treating with saliva.

To mimic the resin composites in oral environment, the composites were aged in distilled water at 37 ^{o}C for 1, 3 and 7 days. The results in Figure 3.6 show that all the composites with AD addition showed no statistically significant changes in CS (Fig.5a), regardless filler loading or aging time. No statistically significant changes in the *S.*

mutans viability were noticed either. This may indicate that most AD derivatives were firmly incorporated into the composites without noticeable leaching.

Finally we measured YS, M, CS, DTS, FS, shrinkage, exotherm and *S. mutans* viability of the resin composites with and without AD addition. As shown in Table 2, the composites with 5-30% AD addition were 7-18% in YS, 0-15% in modulus, 0-2% in CS, 3-22% in DTS, 13-22% in FS and 16-68% in the S. mutans viability lower but 0.13-1.38 times in shrinkage and 0.14-0.56 times in exotherm higher than the composite without AD. All these changes can be attributed to the AD addition. Unlike those dimethacrylates used in dental resin composites, AD is a monoacrylate, which can lead to a decrease in strengths. With increasing AD, shrinkage and exotherm were increased, which can be attributed to the increased quantities of carbon-carbon double bonds (C=C) from AD.

3.6 Conclusions

The dental restorative material developed and evaluated is a novel furanone-containing antibacterial glass-ionomer cement. All of the furnanone-modified cements show significant antibacterial activity, along with an initial reduction in CS strength. DGEGM loading had a significant effect on both antibacterial and mechanical strength. With an increase in loading, the cement had enhanced antibacterial activity, but an initial reduced CS. Modifying the cement with DGEGM showed a broad range of antibacterial activity, killing *S. mutans, lactobacillus, S. aureus,* and *S. epidermidis*. When coated with human saliva, the antibacterial power of the cement was not affected. The long-term aging study indicated that the cements have a long-lasting antibacterial function. Because of the antibacterial function and strength properties, it appears that the furanone-containing glass ionomer is a clinically attractive dental restorative.

4. CONCLUSIONS

We have developed a novel furanone-containing antibacterial dental restorative system with permanent antibacterial function. The furanone-containing glass-ionomer cement showed a significant antibacterial activity, accompanying with an initial CS reduction. The furanone-constructed resin composite showed a significant antibacterial activity without substantially decreasing the mechanical strengths. For GIC, increasing loading significantly enhanced antibacterial activity but reduced the initial CS of the formed cements. The derivative showed a broad antibacterial spectrum on bacteria including *S. mutans, lactobacillus, S. aureus* and *S. epidermidis*. For resin composite, with 5 to 30% addition of the furanone derivative, the composite kept its original CS unchanged but showed a significant antibacterial activity with a 16-68% reduction in the *S. mutans* viability. Human saliva did not affect the antibacterial activity of both furanone-containing GIC and resin composite. The long-term aging study indicates that both antibacterial GIC and resin composite may have a long-lasting antibacterial function. Within the limitations of this study, it appears that both experimental cement and resin composite are clinically attractive dental restoratives that can be potentially used for long-lasting restorations due to their high mechanical strength and permanent antibacterial function.

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